

APPENDIX A



MAR 01 2004

PATENT

Attorney Docket No.: 27373/39055B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Wang *et al.*

Application No. 09/748,710

Filed: December 22, 2000

For: METHOD FOR
GENERATION OF LONGER
cDNA FRAGMENTS FROM
SAGE TAGS FOR GENE
IDENTIFICATION

Group Art Unit: 1637

Examiner: Joyce Tung

) I hereby certify that this correspondence is
) being deposited with the U.S. Postal Service
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) Date: February 19, 2004

) William K. Muehl

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2/18/04

**DECLARATION OF SAN MING WANG, JIAN-JUN CHEN, AND JANET ROWLEY
UNDER 37 C.F.R. § 1.131**

We, San Ming Wang, Jian-jun Chen, and Janet Rowley, state that,

1. We are the inventors of the subject matter claimed in the above-identified application, which claims priority to U.S. Provisional Application Nos.: 60/174,391 and 60/173,617, filed January 3, 2000, and December 29, 1999, respectively. We are making this declaration to provide evidence that the subject matter claimed in the above-identified application was completed in the United States at a date at least prior to July 19, 1999, which is the date of acceptance of van den Berg, et al., Nucleic Acids Research, 1999, Vol. 27(17), pages i-iii, cited by the Examiner in an Office Action mailed March 26, 2003.

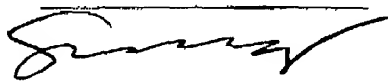
2. To establish the date of completion of the invention, copies of pages from San Ming Wang's laboratory notebook are attached as Exhibit A. All dates appearing on these laboratory notebook pages have been redacted.

3. The laboratory notebook pages establish the following facts:

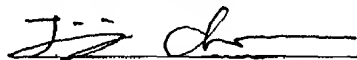
- (a) Information from SAGE tags alone resulted in problems in identifying some genes;
- (b) The solution to the problems associated with SAGE tags was to extend the SAGE tag sequence to generate a longer, more specific 3' cDNA probe;
- (c) The longer probe could be generated using a SAGE tag as a primer in an extension and/or amplification reaction(s);
- (d) One amplification reaction useful in solving the SAGE tag problem is PCR, using a SAGE tag primer and some form of universal primer, such as an oligo (dT) primer, optionally containing an anchor, with a cDNA as a substrate;

4. These laboratory notebook pages confirm that the subject matter of the pending claims in the application was completed at least prior to July 19, 1999.

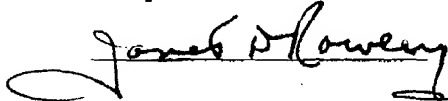
5. Each of us hereby declares as follows: All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true and, further, these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



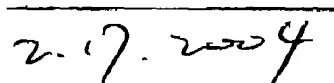
San Ming Wang, Ph.D.



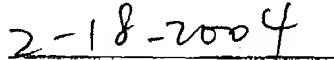
Jian-jun Chen



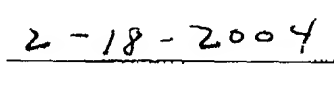
Janet Rowley



Date

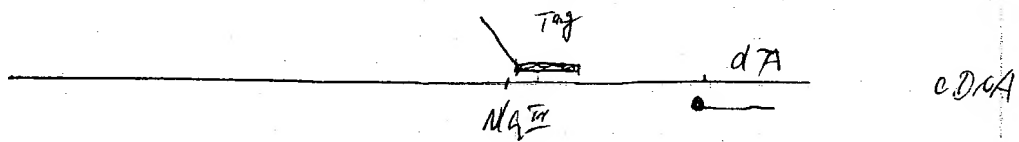


Date



Date

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- based on Tag seq. design 5' seq sense primer, plus 6 bases tail to maintain the stringency

anchored dT

It is known well

Tag pol
nicely distinguish
e anchored primer
reaction

- use anchored primer as 3' antisense primer to select 3' subpopulation for PCR. IMPORTANT!

NEW IDEA

- PCR amplify cDNA
- the cDNA should be pre-digested by Nla^{III}.

The possible outcome would be

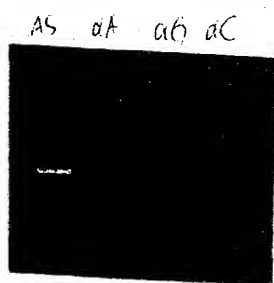
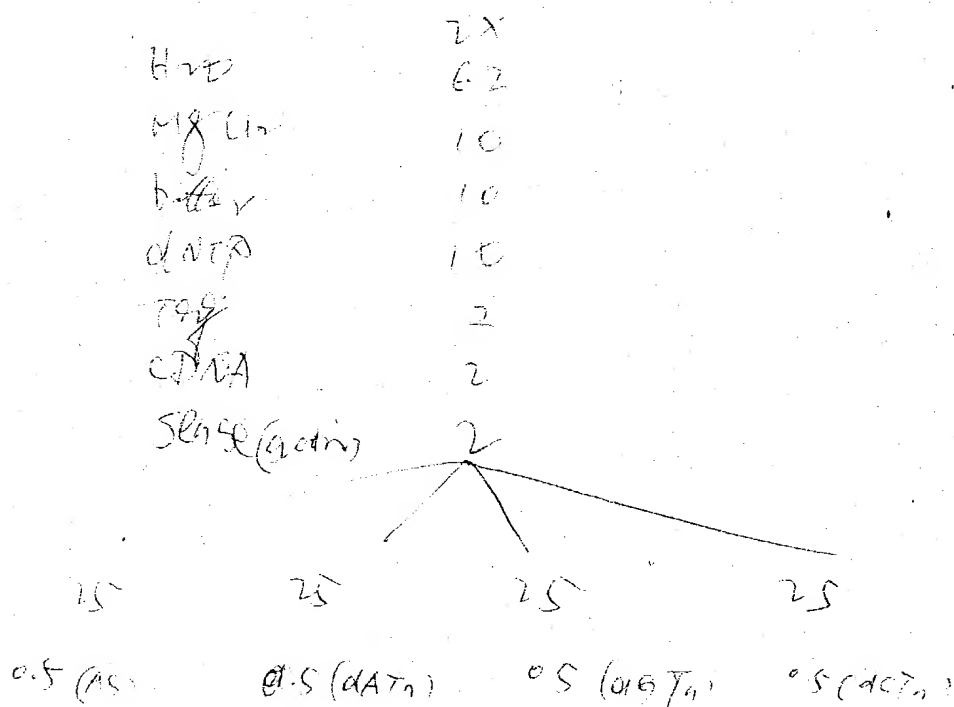
- specific temp amplified because of the 5' sense primer / 3' anchored primer gives exponential amplification, others will only be double increased. 1, 2, 3, 4, ...

potential problem ~

- Amplification efficiency lower due to 3' extension consume large portion of substrates

- specificity?

The possible reason for failure to ~~amplify~~ amplify may be that the mixture of α -actin dT is disturbed the efficient amplification. Try to use separated α -actin dT as 3' primer, use β -actin as model



β -actin mRNA end (HSACGT)

HAAGTG CACAC CTTA TTTTTT
 A TTTTTT (dATn)

Conclusion (1) α -actin as 3' OK

improve:

- increase 3' anchor primer amount
- decrease first 5 cycles in PCR to allow 3' primer bind to temp, then shift to 60°C for annealing
- increase cycle number to 40 Actin

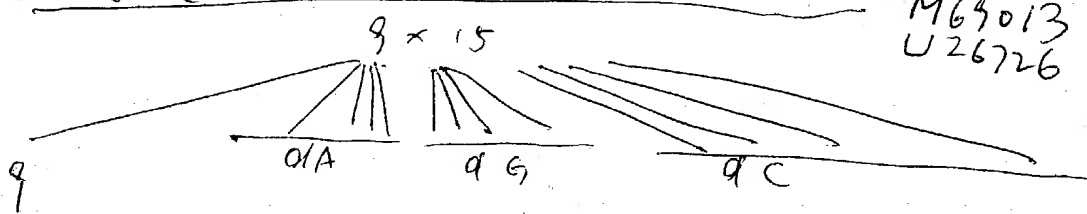
	8x
MyC/2	40
buffer	40
QNEP	40
Taq	8
cDNA	8
Sense	8

def1-2
 ribosomal
 (3) x
 S24

dATn
 dTn
 dCTn

X037K7
 M64013
 U26726

AA5142
 AJ193161
 S28
 AA67692
 AA617970
 AJ223473
 D25786



disease 1
 1 5 10 15 15 10 15 1 5 10 15
 50ng 250ng 500ng 750ng

no 14 14 10 5 - 14 10 5 - 14 10 5 -

25ml

94°C 20"
 42°C 20"
 72°C 20"

94°C 20"
 60°C 20"
 72°C 20"

} x5 → } x30

S/AS
 50ng 100ng 500ng 750ng



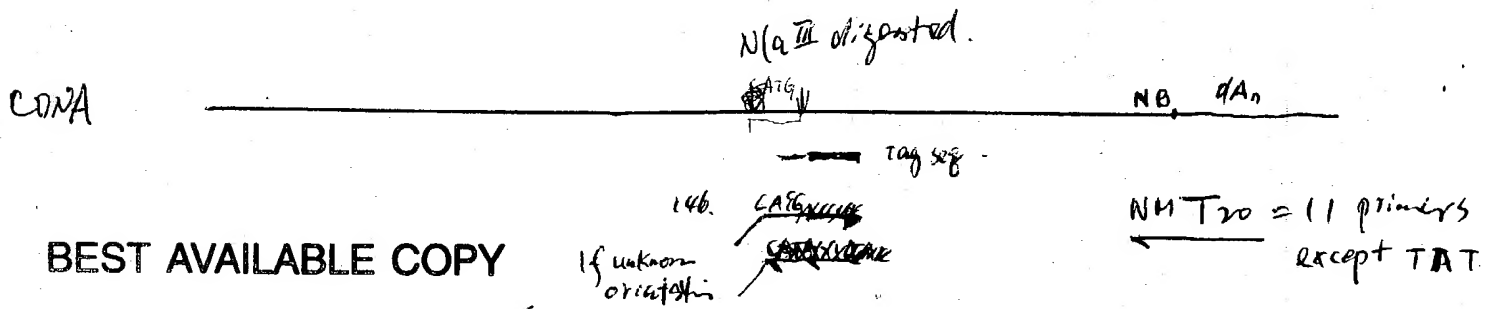
Conclusion:

- ① increase anchor primer amount did increase the amplification efficiency
- ② CTn still generated diffused in principle, this strategy should work! Can be use

* Also, the cDNA should be generated with anchor set, not oligo dT which creates background in PCR.

It seems that one base anchored dT primers may not generate enough specificity in particular temp, due to the complicated cDNA pool composition, or different members in the same family like Hsp70s.

To increase the specificity, it may be useful to use 2 base anchored dT, set as 3' antisense primer. The complete set of sense / antisense will be



- To identify each ~~temp~~ tag gene, the total reaction will be 11 PCR reaction with Tag seq as Sense (+ 6 base ^{5'} tag).
- If negative, reversed Tag sequence will be used as the Sense primer in case the original tag was generated in 3'-5' direction.
- In this way, the specific 3' fragment will be generated, sequenced, and matched to database.

t. try repeat exp. with anchor primer generated cDNA → {one base anchor 5' 5'.